

Sceloporus. This difference between *S. scalaris* and other sceloporine lizards may lie in ecological attributes. *Sceloporus scalaris* inhabits grass clumps whereas most other sceloporine lizards are geophilic. This habitat difference may be enough to eliminate the majority of insect intermediate hosts from the diet as well as remove the lizard from areas contaminated by feces. Further investigation of the helminths of this species would be appropriate.

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Literature Cited

Baker, M. R. 1987. Synopsis of the Nematoda parasitic in amphibians and reptiles. Memorial University of Newfoundland, Occasional Papers in Biology 11:1-325.

Goldberg, S. R., and C. R. Bursey. 1990. Prevalence of larval cestodes (*Mesocostoides* sp.) in the western fence lizard, *Sceloporus occidentalis biseriatus* (Iguanidae), from southern California. Bulletin of the Southern California Academy of Sciences 89: 42-48.

Olsen, O. W. 1974. Animal Parasites. Their Life Cycles and Ecology. University Park Press, Baltimore, Maryland. 562 pp.

Schmidt, G. D. 1986. Handbook of Tapeworm Identification. CRC Press, Boca Raton, Florida. 675 pp.

Stebbins, R. C. 1985. A Field Guide to Western Reptiles and Amphibians. Houghton Mifflin Company, Boston, Massachusetts. 336 pp.

Appendix 1

Sceloporus scalaris examined from Angelo State Natural History Collection: 10728-10735, 11019, 11348-11351, 11358, 11360-11369, 11382, 11383, 11387, 11390, 11392-11394, 11492, 11499, 11609, 11612-11615.

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Research Note

Helminths of the Marine Toad, *Bufo marinus* (Anura: Bufonidae), from American Samoa

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ABSTRACT: The gastrointestinal tracts, lungs, livers, and urinary bladders of 97 *Bufo marinus* were examined for helminths. The nematode, *Parapharyngodon kartana*, was recovered from 1 toad (prevalence 1%). This occurrence represents a new host record. The trematode, *Mesocoelium monas*, was also recovered; prevalence 100% (mean intensity 101) from *B. marinus* collected on Tutuila Island and prevalence 80% (mean intensity 19) for toads from Aunu'u Island. This finding extends the range of *M. monas* to the Pacific Islands.

KEY WORDS: Trematoda, *Mesocoelium monas*, Nematoda, *Parapharyngodon kartana*, Bufonidae, *Bufo marinus*, prevalence, intensity, American Samoa.

The marine toad, *Bufo marinus* (Linnaeus, 1758), originally ranged from southern Texas to central Brazil (Zug and Zug, 1979), but was introduced to many areas including the Caribbean Islands, Pacific Islands, and Australia (Easteal,

1981). It was introduced into Tutuila Island, American Samoa, from Hawaii in 1953 to control insects and later to Aunu'u Island. The population on Tutuila Island was estimated to be 2,296,000 in 1976 by Amerson et al. (1982). To our knowledge, the helminth fauna of *B. marinus* from American Samoa has not been investigated. The purpose of this report is to present findings of an examination of *B. marinus* from American Samoa for helminths.

Ninety-seven *B. marinus* were examined. Eighteen (mean snout-vent length [SVL] 89.5 mm, range 53-127 mm) were collected April-May 1989 from Tutuila Island, American Samoa (14°17'S, 170°41'W); 23 (mean SVL 91.7 mm, range 75-122 mm) were collected January 1990 also from Tutuila Island. Fifty-six (mean

SVL 77.6 mm, range 20–111 mm) were collected January 1990 from Aunu'u Island (14°17'S, 170°33'W). The 1989 sample was examined for cestodes and nematodes; the 1990 sample was examined for cestodes, nematodes, and trematodes. Specimens of the toads were deposited in the herpetology collection of the Los Angeles County Museum of Natural History (LACM): Tutuila Island, 138688–138728; Aunu'u Island, 138729–138784. The body cavity was opened and the esophagus, stomach, and small and large intestines were removed to a dissecting pan, slit longitudinally, and examined under a dissecting microscope. The surfaces of the liver, body cavity, lungs, and urinary bladder were also examined for helminths. Each helminth was identified utilizing a glycerol wet mount. Representative helminths were deposited in the U.S. National Parasite Collection, USDA, ARS, Beltsville, Maryland 20705: *Parapharyngodon kartana* (81917) and *Mesocoelium monas* (81918).

Mesocoelium monas (Rudolphi, 1819) was found in both Tutuila and Aunu'u Island populations of *B. marinus*. In American Samoa, *B. marinus* is restricted to Tutuila and Aunu'u Islands (Amerson et al., 1982). Prevalence of *M. monas* was 100% (23/23), mean intensity 101, range 2–696 in the Tutuila Island population, and 80% (45/56), mean intensity 19, range 1–148 in the Aunu'u Island population. There were significant differences in prevalence and mean intensity of *M. monas* between island populations ($\chi^2 = 5.24$, 1 df, $P < 0.05$ and 56.03, 1 df, $P < 0.001$, respectively). This difference may relate to the relative abundance of sigmurethrid snails (see Fischthal and Kuntz, 1967) on the 2 islands, but to our knowledge a study of relative abundance of Samoan land snails has not been published.

Freitas (1963) has synonymized 19 species of the genus *Mesocoelium* from a wide variety of amphibians and reptiles with *M. monas*. The list of synonymized species was expanded to 23 by Nasir and Diaz (1971) who in the process recognized only 4 species. Nasir and Diaz (1971) believed only 2 characters, the sucker ratio and egg size, are necessary for species determination. We have identified our specimens as *M. monas* because they have a sucker ratio of 1:1 and eggs that average 35 μ m in diameter. *Mesocoelium monas* has been recovered from *B. marinus* from widely separated geographical regions such as Brazil, Colombia, Costa Rica, Hawaii, Paraguay, and Puerto Rico (Nasir and Diaz, 1971). Our

findings extend the range of this parasite to the Pacific Islands.

Five female nematodes, which we identified as *Parapharyngodon kartana* (Johnston and Mawson, 1941) (prevalence 6%, 1/18), were found in 1 of the toads collected in 1989. No nematodes were found in the 1990 sample; thus, by combining the samples, prevalence is reduced to 1% (1/97). Although no male nematodes were recovered, we base our identification on a sample of *P. kartana* taken from the skinks, *Emoia nigra* and *Emoia samoense*, also collected on Tutuila Island in 1990 (Goldberg and Bursey, 1991). The measurements of the specimens from *B. marinus* were within the range of those reported by Angel and Mawson (1968) and are identical to the nematodes we recovered from *E. nigra* (Goldberg and Bursey, 1991). Currently 33 species of *Parapharyngodon* are recognized and various species have been recovered from hosts in 11 families of lizards, 2 of snakes, and 2 of frogs (Baker, 1987). Given the cosmopolitan distribution of the genus and its absence in bufonid amphibians, we believe its occurrence in 1 toad to be a case of pseudoparasitism. Because *B. marinus* is sympatric with *E. nigra* and *E. samoense*, it is possible that skinks may occasionally be taken as prey.

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Literature Cited

- Amerson, A. B., Jr., W. A. Whistler, and T. D. Schwaner. 1982. Wildlife and Wildlife Habitat of American Samoa. II. Accounts of Flora and Fauna. United States Department of Interior, Fish and Wildlife Service, Washington, D.C. 151 pp.
- Angel, L. M., and P. M. Mawson. 1968. Helminths from some lizards mostly from South Australia. Transactions of the Royal Society of South Australia 92:59–72.
- Baker, M. R. 1987. Synopsis of the Nematoda parasitic in amphibians and reptiles. Memorial University of Newfoundland, Occasional Papers in Biology 11:1–325.
- Easteal, S. 1981. The history of introductions of *Bufo marinus* (Amphibia: Anura); a natural experiment in evolution. Biological Journal of the Linnean Society 16:93–113.
- Fischthal, J. H., and R. E. Kuntz. 1967. Digenetic trematodes of amphibians and reptiles from Fiji, New Hebrides and British Solomon Islands. Proceedings of the Helminthological Society of Washington 34:244–251.
- Freitas, J. F. T. 1963. Revisão da família Mesocoelii-

- dae Dollfus, 1933 (Trematoda). *Memórias do Instituto Oswaldo Cruz* 61:177–311.
- Goldberg, S. R., and C. R. Bursey.** 1991. *Parapharyngodon kartana* in two skinks, *Emoia nigra* and *Emoia samoense* from Samoa. *Journal of the Helminthological Society of Washington* 58:265–266.
- Nasir, P., and M. T. Diaz.** 1971. A redescription of *Mesocoelium monas* (Rudolphi, 1819) Freitas, 1958, and specific determination in genus *Mesocoelium* Odhner, 1910 (Trematoda. Digenea). *Rivista di Parassitologia* 32:149–158.
- Zug, G. R., and P. B. Zug.** 1979. The marine toad, *Bufo marinus*: a natural history resumé of native populations. *Smithsonian Contributions to Zoology* 284:1–58.

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Research Note

Cryopreservation of Infective Third-stage Larvae of *Strongyloides ratti*

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ABSTRACT: Infective third-stage larvae of *Strongyloides ratti* were successfully cryopreserved using a modification of the procedure developed for *Strongyloides stercoralis*. The larvae were frozen in a mixture of DMSO and dextran (10% of each in water) in the vapor phase of liquid nitrogen. Cryopreserved larvae were thawed into RPMI-1640 cell culture medium, and incubated overnight in an invertebrate saline to allow time for injured worms to die. The surviving larvae accounted for only 1% to 10% of those frozen, but when they were injected into rats a patent infection was produced.

KEY WORDS: *Strongyloides ratti*, cryopreservation.

Strongyloides ratti, a parasite of rats, is a frequently used laboratory model for species of *Strongyloides* that infect domestic animals and man. Because the rat eliminates an *S. ratti* infection in 3 to 4 wk, it is expensive to maintain this parasite, especially if more than 1 strain is being used. In fact, when we decided recently to re-establish this parasite in our laboratory, we were unable to find a laboratory in the United States that was maintaining it. Although both *Strongyloides stercoralis* (Nolan et al., 1988) and *Strongyloides papillosus* (Van Wyk et al., 1977) have been cryopreserved, the latter was not infective for its host (sheep) upon thawing. Therefore, this investigation was undertaken to determine whether our method for freezing *S. stercoralis* would not only cryopreserve *S. ratti*, but also maintain its infectivity.

The strain of *S. ratti* used in this investigation was G-60 (given to us by Dr. M. E. Viney, University of Edinburgh, Edinburgh, Scotland). This

is a heterogonic strain originally isolated from a wild rat by Dr. G. Graham at the University of Pennsylvania. In our laboratory this strain was maintained in both Wistar rats and multimammate rats (*Mastomys natalensis*). Third-stage infective larvae (L₃) were obtained from 7-day-old coprocultures by baermannization, and washed twice in distilled water. These larvae were held in the freezing medium described for *S. stercoralis* (10% DMSO and 10% dextran; Nolan et al., 1988) for 15 to 90 min, depending on the experiment. They were then frozen in the vapor phase of liquid nitrogen and stored there for 7 to 330 days. The length of time spent frozen had no effect on the survival of the larvae, as was also described for other parasitic nematodes (Nolan et al., 1988; Van Wyk et al., 1990).

The larvae were thawed as described (Nolan et al., 1988) for *S. stercoralis* and were then resuspended in BU, a buffered saline designed for invertebrates (Hawdon and Schad, 1991). It is important to wash the larvae several times after thawing in order to remove all of the freezing medium since it is slightly toxic to the L₃'s. Counts made 30 min after thawing showed no significant difference in survival (as judged by movement) between larvae frozen after incubation at room temperature for 15 to 90 min (Fig. 1). However, when the same larvae were counted approximately 20 hr after being thawed, survival had decreased significantly in all groups, but significantly more survived in the group given a 60-min incubation than in either the 15- or 30-min